REMARKS

1. The haematopoietic stem cells limitation is added to Claim 1 as the Office communication suggested. The amendment is supported by the specification. " ...the purification of these adherent cells was achieved by removal of the haematopoietic stem cells and non-adherent cells during the following changes of medium." [0039]

2. The limitation of "recovering with trypsin-EDTA" previously added in Claim 1 is to distinguish the invention from the method using Leukosorb filter, which recovers cells via elution from the filter using a buffer containing sodium citrate. However, Caplan used trypsin-EDTA to detach cells from the culture dishes but not to elute cells from Leukosorb. This application is different from the combination of Caplan's, Prockop's and Matsui's teachings.

3. Caplan et al teach growth of mesenchymal stem cells without differentiation, and Procokop et al teach that low density culture solves the prior art problems and improved proliferation of mesenchymal stem cells without differentiation. However, Kato et al (US Patent Application 20050013804, filling date: 09/12/2001) still mentioned that "The conventional culture methods however cannot produce sufficient amounts of mesenchymal stem cells because the proliferation of said stem cells stops or becomes extremely slow around 15 th generation." Moreover, this application disclosed that "in one preferred embodiment of the present invention, the isolated MSCs proliferate without differentiation and reach confluence even after 12 passages. The cell populations having greater than 98% homogeneous MSCs are obtained in accordance with the method of the present invention."[0031] This application also added theses "unexpected results" in claims 43-45. (MPEP 2145 & 716.02)

4. 37 CFR 1.57 Incorporation by reference indicates "(a) Subject to the conditions and requirements of this paragraph, if all or a portion of the specification or drawing(s) is inadvertently omitted from an application, but the application contains a claim under § 1.55 for priority of a prior-filed foreign application, or a claim under § 1.78 for the benefit of a prior-filed provisional, nonprovisional, or international application, that was present on the filing date of the application, and the inadvertently omitted portion of the specification or drawing(s) is completely contained in the prior-filed application, the claim under § 1.55 or § 1.78 shall also be considered an incorporation by reference of the prior-filed application as to the inadvertently omitted portion of the specification or drawing(s). " On pages 9 and 11 of this certified copy of foreign priority application of this application, we did disclose the pore size is around 0.4 to

16 microns. These two pages are attached again for reference. Therefore, the claim 42 to limit the pore size to be 0.4 to 16 microns in diameter shall not induce new matter.

Accordingly, this application should be placed in condition of allowance. An early Notice to this effect is respectfully expected.

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表面抗原以及相關的細胞活性可能因此受到影響甚至損壞，此外，使用專一性的單株或多株抗體，亦會使得分離的成本及時間增加。因此，對於發展一種簡易、安全、有效並經濟之分離骨髓及其他來源的間質幹細胞的方法，以大量用於相關疾病的治療，有其急迫的需要。

發明概述

有鑑於此，本發明提供一種分離間質幹細胞之方法，包括：（a）提供一包含間質幹細胞之細胞混合物；（b）將該細胞混合物接種至一培養裝置上；以及（c）回收並培養該間質幹細胞。較佳地，此培養裝置包括一具有孔洞（例如，孔徑大約0.4到16微米）之膜板，其中該孔洞足以分離間質幹細胞以及其他細胞。

本發明的第二形態是提供一種分離的間質幹細胞，係藉由上述方法而分離，此分離的間質幹細胞具有複製更新及多分化之功能。

本發明的第三形態是提供一種組成物，包括上述之分離的間質幹細胞以及一培養液，其中該培養液可使該間質幹細胞生長。

本發明更包括一種醫藥組合物，包括上述之分離的間質幹細胞以及一藥學上可接受之載體，其中，當組織因老化、外傷或疾病損壞時，該間質幹細胞可作為細胞置換及基因治療之用途。

為了讓本發明之上述和其他目的、特徵，及優點能更明顯易懂，下文特舉較佳實施例並配合附件，做詳細說明

細胞（progenitor），包括，但並不限於，脂肪、軟骨、骨骼以及纖維結締組織。有一些骨髓來源的間質幹細胞之表面標幟，可被一些單株抗體辨認，例如，SH2、SH3及SH4（參見美國專利第5,965,436號）。然而，對於免疫篩選的方法，仍是缺乏專一性的標幟以用於分離間質幹細胞。即使是以抗-CD41單株抗體結合巨核球細胞，以間接分離間質幹細胞的方法，其多項步驟所需之成本以及所分離的細胞之純度，仍有改進的空間。

因此，本發明提供一種新穎、簡易、有效且經濟之分離間質幹細胞的方法，包括下列步驟：（a）提供一包含間質幹細胞之細胞混合物；（b）將該細胞混合物接種至一培養裝置上；以及（c）回收並培養該間質幹細胞。

本發明之分離方法是根據細胞大小的不同、細胞附著能力的不同，以及當與$CD34^+$造血幹細胞共同培養時，間質幹細胞僅是扮演支持角色等的特性，而將間質幹細胞從細胞混合物中分離出來。由於間質幹細胞較其他的骨髓細胞大（van Vlasselaer P, et al., supra）、易於附著於培養皿表面，以及支持造血幹細胞的角色（Huang S., et al., Nature 360:745, 1993）之因素，本發明可使用一種培養裝置而將間質幹細胞物理性地分離出來。

在一較佳具體實施例中，上述用以分離細胞之培養裝置包括一具有孔洞之膜板，其中孔洞的大小足以分離間質幹細胞以及其他細胞。更佳地，此孔洞具有大約0.4到16微米（μm）範圍之直徑。